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The Effect of a High Polyunsaturated, High Fiber, Lacto-Vegetarian Diet on the Serum Cholesterol and Serum Triglycerides in Human Subjects

Linda Johnson

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Abstract

THE EFFECT OF A HIGH POLYUNSATURATED, HIGH FIBER, LACTO-VEGETARIAN DIET ON THE SERUM CHOLESTEROL AND SERUM TRIGLYCERIDES IN HUMAN SUBJECTS

by Linda Johnson

Fifteen male high risk coronary subjects were fed a lacto-vegetarian diet for 28 days. The diet was calculated with a P:S ratio of 4.0 or greater and the subjects received at least 10 g. of fiber per day. At weekly intervals, the blood was drawn and serum cholesterol and triglyceride determinations were done. As a result of the diet, the men experienced a significant decrease in cholesterol ($p>.001$). During the 28 days, there was a 29 per cent decrease in mean serum cholesterol and a 40 per cent decrease in mean serum triglycerides.

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THE EFFECT OF A HIGH POLYUNSATURATED, HIGH FIBER, LACTO-VEGETARIAN DIET ON THE SERUM CHOLESTEROL AND SERUM TRIGLYCERIDES IN HUMAN SUBJECTS

by

Linda Johnson

A Thesis in Partial Fulfillment of the

Requirements for the Degree

Master of Science in the Field of Nutrition

March 1976

Each person whose signature appears below certifies that this thesis in his opinion is adequate, in scope and quality, as a thesis for the degree Master of Science.

James W. Blankenship, Chairman
James W. Blankenship, Associate Professor

U. D. Register, Professor of Nutrition

Irma B. Vyhmeister, Associate Professor of Nutrition

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THE EFFECT OF A HIGH POLYUNSATURATED, HIGH FIBER, LACTO-VEGETARIAN DIET ON THE SERUM CHOLESTEROL AND SERUM TRIGLYCERIDES IN HUMAN SUBJECTS

INTRODUCTION

Coronary heart disease is a major public health problem in the United States and one of the predisposing risk factors for heart disease is elevated serum lipid levels (Am. J. Clin. Nutr., 1973; Med. J. Aust., 1974). A program which decreases the serum cholesterol and serum triglyceride levels has shown to be an effective treatment for decreasing the risk of coronary heart disease or atherosclerosis (Gotto and Scott, 1973). An excellent example was reported by Gotto et al. (1974) where a 10 per cent reduction in cholesterol alone yielded a 23 per cent decrease in the incidence of coronary heart disease.

Although there are other factors affecting the serum lipids, dietary habits are the easiest to manipulate and are most highly associated with the development of atherosclerosis and coronary heart disease. Dietary fats and carbohydrates, which total approximately 85 per cent of the average American diet, have been noted as the major factors influencing blood lipids (McGandy et al., 1967). Other factors important to the control of serum cholesterol and triglycerides are dietary cholesterol, fiber, and alcohol.

CHOLESTEROL

Elevated serum cholesterol levels have been regarded by many to be the major risk factor in coronary heart disease. The Framingham study

stated risks for coronary heart disease at five times higher in patients with serum cholesterol levels above 260 mg. per 100 ml. as compared to those with values below 220 mg. per 100 ml. (Gotto et al., 1974). This has led to the conclusions that serum cholesterol should remain below 200 mg. per 100 ml. throughout life (Med. J. Aust., 1974; Albrink, 1974).

Cholesterol is absorbed with other lipids as it is transported across the intestinal wall. Once absorbed, cholesterol enters the endoplasmic reticulum where it forms a complex with protein, fat and phospholipids to form chylomicrons. The lipid in the form of triglycerides, cholesterol, and cholesterol esters can be transported to the tissues in the chylomicron (Masoro, 1968). In the serum, there is a constant combination of biosynthetic and dietary cholesterol until they mix and become indistinguishable (Connor and Lin, 1974).

Dietary Cholesterol and Serum Cholesterol

Forty per cent of serum cholesterol in man is from dietary sources while the remainder is the result of body synthesis (Med. J. Aust., 1974). The body synthesizes approximately one gram of cholesterol per day. Even though body synthesis of cholesterol continues while one is on a low cholesterol diet, the body will not produce cholesterol above what is taken away in the diet; therefore, the serum levels continue to drop (Stamler, 1974). The biosynthetic cholesterol from both the liver and the intestinal mucosa remain generally constant. It is evident that dietary cholesterol is additive and that serum cholesterol levels may be altered by reducing or increasing amounts of cholesterol in the diet (Connor and Lin, 1974).

Approximately 45 per cent of the cholesterol ingested is absorbed (Connor and Lin, 1974). Serum cholesterol and dietary cholesterol form a linear relationship with the absorption of cholesterol increasing proportionately with increases in the dietary cholesterol (Connor and Lin, 1974). The results of one study show that for each 100 mg. cholesterol per 1000 calories ingested, there was an increase of 12 mg. per 100 ml. in the serum cholesterol (Med. J. Aust., 1974). The level of cholesterol restriction suggested by Albrink (1974) was 300 mg. per day or less. Levy (1972) reports that the average American consumes a diet rich in cholesterol (600-800 mg. per day). To reduce elevated serum cholesterol, one should use a diet containing less than 300 mg. per day. Wilson et al. (1971) observed that by restricting the intake of dietary cholesterol, the serum cholesterol was lowered.

In a more recent study, Connor et al. (1964) found more changes in serum cholesterol when the dietary cholesterol was manipulated than when the fats were modified. When cholesterol was removed from the diet for four weeks, serum cholesterol decreased by 38 mg. per 100 ml. even with a large quantity of saturated fat (100 g. per day). The next four weeks, dietary cholesterol was added (725 mg.) resulting in an increase in serum cholesterol in spite of large amounts of polyunsaturated fats present (90 g. of the total 100 g. fat were polyunsaturated).

It is evident, that while dietary cholesterol does have a direct effect on serum cholesterol levels, the absorption of cholesterol depends upon other lipids. Some studies obtained results in controlling hypercholesterolemia with dietary cholesterol restrictions. Others feel the polyunsaturated to saturated fat ratio (P:S ratio) is more significant

than any amount of dietary cholesterol (Gotto and Scott, 1973). The P:S ratio has become an important element in controlling hypercholesterolemia.

Dietary Fats and Serum Cholesterol

Many investigators believe that the serum cholesterol reflects the fat intake rather than the cholesterol intake (Mellinkoff et al., 1950; Morrison et al., 1950; Hildreth et al., 1951). The lowering of fat in the diet below 40 g. per day tends to lower serum cholesterol concentration (Ahrens et al., 1957). Wilkens et al. (1962) reports that it is only logical to restrict fats to a minimum, especially when the normal amount of fat in the American diet equals approximately 40 per cent of the total calories (Wilson et al., 1971).

The type of fat in the diet will influence the serum cholesterol concentration. A high intake of saturated fats usually results in increased serum cholesterol levels (Med. J. Aust., 1974; Bronte-Stewart et al., 1956; Ahrens et al., 1957). Conflicting results, however, have been reported where serum cholesterol levels were not increased with high levels of saturated fats (Gordon, 1958; Ahrens et al., 1957).

A diet with more fat than 30-35 per cent of the calories may lower lipids if the fat is polyunsaturated. Studies in South Africa by Bronte-Stewart et al. (1956) showed that sunflower seed oil and other highly unsaturated fats consistently result in decreased serum cholesterol. This was true when the unsaturated fats are fed alone, with a cholesterol supplement, or with an animal fat supplement. Ahrens et al. (1957) used a liquid formula diet resulting in lower serum cholesterol from ingesting highly unsaturated oils. Another study by Ahrens et al. in 1955 showed

that serum cholesterol was lowest using corn oil, rather than olive, lard, or coconut oil. He concluded that the reduction in serum cholesterol depends on the fat fed, rather than on the decreased intake of cholesterol (Ahrens et al., 1955).

More recent studies also show similar results. The American Heart Association diet, which is low in saturated fat, low in cholesterol, moderate in carbohydrates, and low in calories, decreased both serum cholesterol and triglycerides by 9.6 and 11.7 per cent respectively (Wilson et al., 1971; Hall et al., 1971). Another study by McGandy et al. (1967) showed serum cholesterol reduced by 12 per cent with a diet low in cholesterol and saturated fats and high in polyunsaturated fats. Hardinge and Stare (1954) showed that pure vegetarians, with free use of vegetable fats, had the lowest serum cholesterol values compared to nonvegetarians and lacto-ovo vegetarians. The reduction in serum cholesterol with a high P:S ratio diet is maximal within two to three weeks and will be sustained (Chait et al., 1974).

The serum cholesterol changes resulting from a high P:S ratio diet seem to be well worth the effort necessary to change dietary habits. Mass dietary changes would be necessary as evidenced by national P:S averages ranging from .2 to .3 (Albrink, 1974; Hodges and Krehl, 1963). The P:S ratio recommended to reduce and maintain low risk serum levels of lipids is 1.5 (Med. J. Aust., 1974).

Dietary Carbohydrates and Serum Cholesterol

Prolonged excesses of calories and simple sugars may lead to hyperlipemia, mainly hypertriglyceridemia (Ahrens et al., 1961). Increased intake of carbohydrates leads to overloading of the Embden-Meyerhof pathway.

The overloading results in increased use of the hexose monophosphate shunt. More $NADPH+H^+$ is produced which enhances the formation of fatty acids and cholesterol (Hodges and Krehl, 1963).

The type of carbohydrate, along with the quantities, are important to the level of serum cholesterol. Numerous studies have shown that serum cholesterol concentrations were lower when complex carbohydrate was eaten such as found in fruits and vegetables rather than sucrose as found in sweetened pies, cakes, cookies and candy (Keys et al., 1960; Lopez et al., 1966; Hodges and Krehl, 1963; McGaney dt al., 1967). Winitz et al. (1964) reported that sucrose is a hypercholesterolemic agent when compared to other simple sugars. More recent literature places sugars in a less effective position when relating them to elevation of serum cholesterol. The changes reported in a switch from simple to complex carbohydrates is very small in comparison to differences resulting from various fats (McGandy et al., 1967). The suggestion has been made that there is no need to limit dietary sucrose in a hypercholesterolemic patient when he is already restricted in dietary cholesterol and using polyunsaturated fats (Birchwood et al., 1970).

Dietary Fiber and Serum Cholesterol

Dietary fiber has an effect on blood lipids (McGandy et al., 1967). A study making use of pectin reported that with a dietary intake of 15 g. per day, the serum cholesterol was reduced significantly within three weeks (Keys et al., 1961). In studies on rabbits fed alfalfa, the serum cholesterol was unaffected when fed dietary cholesterol along with the alfalfa. When the alfalfa was replaced with regular calf meal, the serum cholesterol shot up rapidly (Cookson et al., 1967). The regular calf meal

pellets contained oats, wheat, soy bean, calcium phosphate, vitamins A, D, and E, salt, beet molasses, and brewers' yeast. A diet with high fiber content reduces serum cholesterol by causing a break in the entero hepatic circulation of bile salts. Dietary fiber binds the cholesterol as it passes through the small intestines, preventing reabsorption. In the absence of reabsorption in the liver, more cholesterol is degraded to bile salts which results in lowering serum cholesterol (Moore, 1967). Cookson et al. (1967) explain that a high residue diet will not improve the atheromatous plaque formations, but will prevent further lesions from forming.

Alcohol and Serum Cholesterol

Literature is varied as to the results of alcohol upon serum cholesterol. In a study where alcohol was given over a period of one week, small increases in serum cholesterol were evident (Losowsky et al., 1963). In a study by Schlierf et al. (1964) using ethanol or very dry wine every one or two hours for 6-18 days, the results showed no elevation of serum cholesterol when polyunsaturated fats were used. Total alcohol consumption per day ranged from 61.6 g. for six of the subjects, down to 19 g. for two subjects. Ingestion of polyunsaturated fats protects against the hyperlipidemic effects of alcohol. Only some of the subjects experienced a marked increase in serum cholesterol concentration when saturated fats were consumed (Schlierf et al., 1969). According to the literature, alcohol seems to have a more consistent hypertriglyceridemic effect which will be covered in the next section.

TRIGLYCERIDES

Evidence strongly points to hypertriglyceridemia as being an independent risk factor for coronary heart disease (Wilson et al., 1971; Hall et al., 1972; Albrink, 1974; Brown et al., 1965; Gotto and Scott, 1973; Masoro, 1968; Carlson and Bottiger, 1972). A combination of both hypertriglyceridemia and hypercholesterolemia carries the highest risk for coronary heart disease (Carlson and Bottiger, 1972). Triglycerides are one of the components of atherosclerotic plaques along with cholesterol esters, phosphoglycerides, sphingolipids, and cholesterol (Masoro, 1968).

Most of the serum triglycerides in the fasting state are present in the very low density lipoprotein (VLDL) or pre-beta lipoprotein fraction. Therefore, the fall in the triglyceride levels during a restrictive diet is due to decreased amounts of VLDL triglycerides. Endogenous VLDL triglyceride fatty acids are derived from plasma free fatty acids as well as from fatty acid synthesis by the liver from carbohydrates. One theory states that the solution to hypertriglyderidemia may be through reducing the free fatty acids which reduces the endogenous VLDL triglycerides (Chait et al., 1974). Chait et al. (1974) suggest that saturated plasma free fatty acids might be incorporated into VLDL triglycerides more easily than unsaturated free fatty acids. Assuming this is valid, then a high P:S ratio diet would reduce the VLDL. VLDL is thought to be a precursor for low density lipoproteins (LDL). The decreased VLDL leads to a reduction in LDL and a resultant decrease in serum cholesterol (Chait et al., 1974).

Dietary Carbohydrates and Serum Triglycerides

Many researchers, such as Yudkin, Roddy, and Morland, feel that there is a reliable statistical relationship between sugar intake and coronary heart disease (Kuo, 1968). It has long been known that dietary carbohydrates can be converted into lipid by the body. Previous to 1968, fat had been regarded as the foremost factor in human atherosclerosis. During 1968, concepts changed when evidence pointed to a disorder in carbohydrate metabolism as the key causative factor in atherosclerotic disease (Kuo, 1968).

Studies have shown that some sugars have a more hypertriglyceridemic effect than others. In rats, fructose has a higher lipaemic effect than glucose. This may be due to the fact that fructose does not stimulate the enzyme lipo-protein lipase which aids in clearing the triglycerides from the blood to the tissues (Bar-On and Stein, 1968). In another study, results insinuated that fructose had a lower lipaemic effect than both sucrose and glucose (McGandy et al., 1967).

Total caloric intake and obesity are of major importance in hypertriglyceridemia (Gotto and Scott, 1973). Carbohydrate rich diets may lead to hypertriglyceridemia, but only when the intake of carbohydrate is exceedingly high. If the carbohydrates total 50 per cent of the calories, the serum triglycerides will usually not be elevated if the calorie level is correct (Med. J. Aust., 1974).

An important study upon human subjects involved administration of an isocaloric, high carbohydrate diet. Serum triglycerides increased in all but one of the sixteen subjects. A few of the patients were continued on the diet for four to ten weeks. The triglyceride levels

decreased, but none of the patients showed full adaptation to the high carbohydrate diet (Mancini et al., 1973).

The early theories on diet and triglycerides conclude that triglycerides are increased by dietary carbohydrates and decreased by calorie restriction and substitution of fat for carbohydrates in the diet (Albrink et al., 1962). Recently, with evidence showing that hypertriglyceridemia may act synergistically with hypercholesterolemia, it does seem realistic that the same restrictions in diet would be effective in reducing both serum lipids (Hall et al., 1972). Recent studies have supported these theories and have shown success in reducing serum triglycerides with a low cholesterol, low saturated fat, and high polyunsaturated fat diet (Chait et al., 1974; Wilson et al., 1971).

Dietary Fats and Serum Triglycerides

Findings show that plasma triglycerides are sensitive not only to an increase in the proportion of dietary carbohydrates, but also to increases in saturated fats (Nestel and Barter, 1973). Many sources state that a high P:S ratio diet has a serum triglyceride lowering effect (Beveridge et al., 1964; Bagdade et al., 1970; Chait et al., 1974; Wilson et al., 1971).

Using the American Heart Association diet, which included a high P:S ratio, triglycerides were reduced by 11.7 per cent after six months (Chait et al., 1974). A long-term study conducted by Hall et al. (1972) reported similar results. Using a diet low in saturated fat and cholesterol, moderate in polyunsaturated fat and carbohydrate and calorie controlled to lower weight, a 17.3 per cent reduction in serum triglycerides was reported after one year. The mean daily nutrients of the diet in

per cent of total calories were: total fat, 30.7 per cent; saturated fat, 9.5 per cent; polyunsaturated fat, 6.7 per cent, carbohydrates, 47.3 per cent. Cholesterol intake was 289 mg. per day and alcohol intake totaled 9 g. per day. The carbohydrates were mainly from grains, fruits, and starchy vegetables. These results support the growing idea that a low carbohydrate diet is seldom required to achieve a significant lowering of serum triglycerides (Hall et al., 1972).

With a high saturated fat diet, both an increased influx of triglycerides from the liver and a reduction in clearing from the serum to the fat ctores are in operation to result in increased serum triglycerides (Nestel and Barter, 1973). A study in rats supports this theory and further reported that lipoprotein lipase was stimulated with a polyumsaturated fat diet (Kohout et al., 1971; Med J. Aust., 1974). Further experiments have shown that polyunsaturated fats, in contrast to saturated fats, are incorporated into glycerides to a lesser extent (Nestel and Barter, 1973; Med. J. Aust., 1974).

A couple of other factors involved in the level of serum triglycerides are body weight and alcohol consumption. Serum triglycerides tend to increase when one gains weight (Albrink et al., 1962; Gotto et al., 1974), and decline when the subject loses weight (Wilson et al., 1971; Hall et al., 1972). Alcohol causes an overproduction of triglycerides in the liver (Med. J. Aust., 1974). Other studies have also reported an increase in serum triglycerides with the use of alcohol (Losowsky et al., 1963; Albrink, 1974).

PURPOSE OF STUDY

A controlled study was conducted to determine the effect of a vegetarian high polyunsaturated fat, high fiber diet on human serum cholesterol and triglyceride levels. Fifteen male subjects with cholesterol levels greater than 250 mg.% were given the cholesterol lowering diet for a period of 28 days. Seven of these subjects also had elevated serum triglyceride levels (> 150 mg.%). Many previous studies support the theory that a low cholesterol, low saturated fat, high polyunsaturated fat diet does reduce serum cholesterol (Hardinge and Stare, 1954; Ahrens et al., 1957; McGandy et al., 1967; Gotto et al., 1974). More recent studies have also reported that this same therapeutic diet will have a triglyceride lowering effect (Bagdade et al., 1970; Wilson et al., 1971; Chait et al., 1974). Since these latter studies are few in number, it seems desirable to re-establish the effectiveness of the cholesterol lowering diet on serum cholesterol and, at the same time, see how effective the diet is in reducing serum triglycerides.

METHODS

Subjects

Fifteen male subjects were selected from a community heart attack risk program (See appendix for questionnaire). The individuals were selected on the basis of age, cholesterol level, and willingness to participate in the scientific study. Age of the men ranged from 28 to 57. Cholesterol levels were above 250 mg.% in all participants and serum triglycerides were elevated (> 150 mg.%) in seven of the 15. All 15 individuals participated in the study the entire 28 days.

The men were served three meals a day, seven days a week during the four-week study. The morning and evening meals were served in the nutrition research kitchen. After breakfast, the subjects were given a prepared sack lunch for the noon meal. After the 28-day study, the men returned to a diet of their own choice for six weeks at which time the men's blood was reanalyzed again for serum cholesterol and serum triglycerides.

A research laboratory in the Nutrition Department was arranged to collect blood samples by a medical technologist and a physician. Each Sunday morning between seven and eight the subjects had a fasting blood sample drawn. Breakfast was served immediately after the blood drawing.

Diet

The individuals were provided a lacto-vegetarian diet during the 28-day study. Polyunsaturated:saturated fat ratio, cholesterol level and fiber content were specifically altered in the diet. Less than 35 per cent of the daily caloric intake was fat with a P:S ratio being 4.0. The main sources of fat included a polyunsaturated corn oil margarine, liquid corn oil and vegetarian meat analogs. No significant source of cholesterol in foods was provided and an egg substitute was used for breakfast and baking.

Fiber was included in the diet at about 10 g. per day. Foods high in fiber, such as fresh fruit and vegetables, whole grain breads and cereals, and textured vegetable protein (TVP) were used. Additional fiber was used by adding bran to cereals, entrees, or desserts to increase the amount of fiber. (See Table 1 for approximately composition of daily menu.)

Table 1

Average Composition of a Daily Menu

Average calories	2500
Percent of calories from fat	35%
Percent of calories from protein	14%
Percent of calories from carbohydrate	51%
Average grams of fiber	10 grams
Mg. cholesterol	insignificant

The menu for the 28 days was a 7-day cycle (See Table 2). The first week began with day one menu and subsequent weeks with day two menu, day three menu, and day four menu. No meal was the same for any given day each week. Once a week a buffet supper with a specific cultural theme was served.

The subjects were not restricted on total food intake except for desserts. The amount of calories was adjusted so persons maintained weight within five pounds of the initial weight. The individuals ate all the meals from the food provided. Water was not limited, but no alcohol was to be consumed during the study.

Each day's diet was adequate in the RDA of nutrients and no supplementation was given. A computerized nutrient analysis was compiled from USDA Handbook #8.

Cholesterol and Triglyceride Determination

Fasting blood was collected in vacuum tubes containing no anticoagulant. After centrifuging the coagulated blood at 2,000 RPM for 15-20 minutes, the supernatant serum was drawn off. Serum was used for both cholesterol and triglyceride determinations.

Cholesterol was determined by a modification of the Liebermann-Burchard reaction where the cholesterol reacts with acetic anhydride, sulfuric acid, and p-toluenesulfonic acid resulting in a blue-green color. A standard cholesterol solution is then used for comparison (Pearson, 1953).

The serum cholesterol was determined by the following procedure: 0.2 ml. of each serum unknown was placed in a cuvette. Added to the serum was 0.2 ml. of glacial acetic acid from a syringe microburette,

Table 2

A Seven-Day Lacto-Vegetarian Menu
for Cholesterol Lowering Study

DAY 1

Breakfast
- Grapefruit half
- Meat analog
- Whole grain cereal, cooked
- Cracked wheat toast

Noon
- Sandwich
  - Whole Wheat bread
  - Meat analog
  - Lettuce, tomato
  - Salad dressing
- Cucumber slices
- Fresh pear
- Applesauce cupcake

Evening
- Cashew Nut Loaf
- Mashed potatoes/gravy
- Carrots
- Tossed salad/French or Italian dressing
- Fruit jello

DAY 2

Breakfast
- Banana
- Granola
- Meat analog
- Cracked wheat toast

Noon
- Chicken-style salad
- Cornbread muffin/margarine
- Fresh orange
- Cranberry crisp

Evening
- Meat analog
- Baked potato/margarine
- Mixed vegetables
- Tossed salad/French or Italian dressing
- Fresh fruit plate

DAY 3

Breakfast
- Orange juice
- Scrambled Egg substitute
- Meat analog
- Cracked wheat toast

Noon
- Sandwich
  - Whole Wheat bread
  - Loma Linda Sandwich Spread
  - Lettuce
  - Salad dressing
- Fresh pear
- Chip oatmeal cookies

DAY 4

Breakfast
- Banana
- Oatmeal/raisins
- Meat analog
- Bran muffin

Noon
- Sandwich
  - Whole Wheat bread
  - Meat analog
  - Lettuce
  - Salad dressing
- Cucumber slices
- Apple pie cake

Table 2 (continued)

Evening
- Stroganoff/brown rice
- Broccoli
- Tossed salad/French or Italian dressing
- Jello parfait

Evening
- Tamale Pie
- Baked potato/margarine
- Green beans
- Coleslaw
- Fresh orange segments

DAY 5

Breakfast
- Apple wheat waffles
- Peanut butter
- Applesauce
- Meat analog

Noon
- Macaroni salad
- Rye Krisp
- Fresh apple
- Fruit bars

Evening
- Spaghetti/meatballs & sauce
- Garlic toast
- Peas
- Tossed salad/French or Italian dressing
- Fruit sherbet

DAY 6

Breakfast
- Grapefruit half
- Brown rice/raisins
- Meat analog
- Cracked wheat toast

Noon
- Sandwich
  - Hard roll
  - Meat analog
  - Lettuce, tomato
- Carrot sticks
- Fresh pear
- Gingersnap cookies

Evening
- SPECIAL MENU
- Chili dogs
- Chinese supper
- Burgers

DAY 7

Breakfast
- Fruit over toast
- Scrambled egg substitute
- Meat analog

Lunch
- Sandwich
  - Meat analog
  - Lettuce, tomato
  - Whole wheat bread
  - Salad dressing
- Green pepper
- Banana
- Peanut butter cookies

1 ml. of 12 per cent p-toluenesulfonic acid in glacial acetic acid, and 3 ml. of acetic anhydride both from an automatic pipette. These solutions then stand at room temperature without mixing until they are cool. Finally, 0.4 ml. of concentrated sulfuric acid were added from a syringe microburette. The contents were mixed immediately by inversion until all the precipitate was dissolved. The solutions were allowed to stand for 20 minutes before reading in a Bausch-Lomb Spectonic 20 colorimeter.

The unknowns were read against a standard prepared with 2 ml. of glacial acetic acid solution with 200 mg. cholesterol per 100 ml. This standard was treated identically to the unknowns with the same additions of 12 per cent p-toluenesulfonic acid in glacial acetic acid, acetic anhydride, and sulfuric acid. Both unknowns and the standard were read at 540 mu after the colorimeter was set at zero against the reagent blank. All serums were run in duplicate and the duplicates show an average deviation of less than 5 per cent (Pearson, 1953).

A reagent blank was prepared by combining 0.2 ml. of water and 0.2 ml. of glacial acetic acid from the syringe microburette. Serum blanks were prepared by adding 4.6 ml. of p-toluenesulfonic acid solution to 0.2 ml. of serum and mixing. The serum blanks were read in the colorimeter which was set at zero by the p-toluenesulfonic acid solution.

Calculations utilized the following formula:

$$\text{Mg. of cholesterol in 100 ml.} = D_s \times \frac{D_u - D_{sb}}{D_s}$$

C_s: Concentration of cholesterol in the standard

D_{sb}: Reading of the serum blank

D_u: Reading of the unknown

D_s: Reading of the standard

Serum was mailed to Lancet laboratories in Portland, Oregon, where triglyceride determinations were completed and results returned by mail.

RESULTS AND DISCUSSION

A lacto-vegetarian diet that was high in polyunsaturated fat (P:S ratio 4.0) and high in dietary fiber (10 g./day) was fed to 15 male subjects for 28 days. All the subjects had serum cholesterol levels greater than 250 mg.% and seven of the 15 had elevated serum triglycerides (> 150 mg.%). Weekly blood samples were drawn and the serum cholesterol and triglycerides were determined. After 28 days on the lacto-vegetarian diet, there was a 29 per cent decrease in mean serum cholesterol (See Table 3) and a 40 per cent decrease in mean serum triglyceride levels (See Figure 1). Although all 15 men participated in the study for the required length of time, only 14 could be used for cholesterol computation. One subject, number 8, used Atromid-S, a cholesterol lowering agent during the first two weeks of the study. Results for this man were very satisfying, however, as after week 2 his serum cholesterol remained low (See Table 3).

Another man, number 2 (See Table 3), against our wishes consumed small amounts of alcohol during the entire study. Alcohol's effect upon serum cholesterol is questionable, but other studies indicate that #2's decrease in serum triglycerides may have been greater without alcohol (Lasowsky, 1963; Albrink, 1974). All 15 subjects were used to compute serum triglyceride data even though only 12 of the 15 decreased serum triglycerides (See Figure 1).

Table 3

Serum Cholesterol Levels Before the Diet and at Weekly Intervals During the 28-day Study

	BEFORE DIET	WEEKS ON DIET			
Subject	Cholesterol (mg. %)	Cholesterol (mg.%)			
	0	1	2	3	4
# 1. C.B.	350	449	232	285	239
*# 2. A.C.	252	235	213	219	176
# 3. W.C.	250	309	207	256	227
# 4. R.G.	296	356	241	233	244
# 5. W.G.	264	275	168	207	207
# 6. H.G.J.	250	287	189	216	177
# 7. H.J.	307	241	197	175	149
**# 8. B.L.	202	236	262	194	201
# 9. H.R.	330	270	257	216	192
#10. O.S.	307	276	243	230	204
#11. V.S.	273	251	168	173	242
#12. F.S.	270	254	207	215	251
#13. R.S.	306	304	207	224	176
#14. P.S.	257	234	197	209	176
#15. J.T.	277	320	216	162	162
Averages	279	287	214	214	202

*Number 2 consumed small amounts of alcohol before and during the diet.

**Number 8 used Atromid-S during the first two weeks on the diet. No medication during weeks 3 and 4.

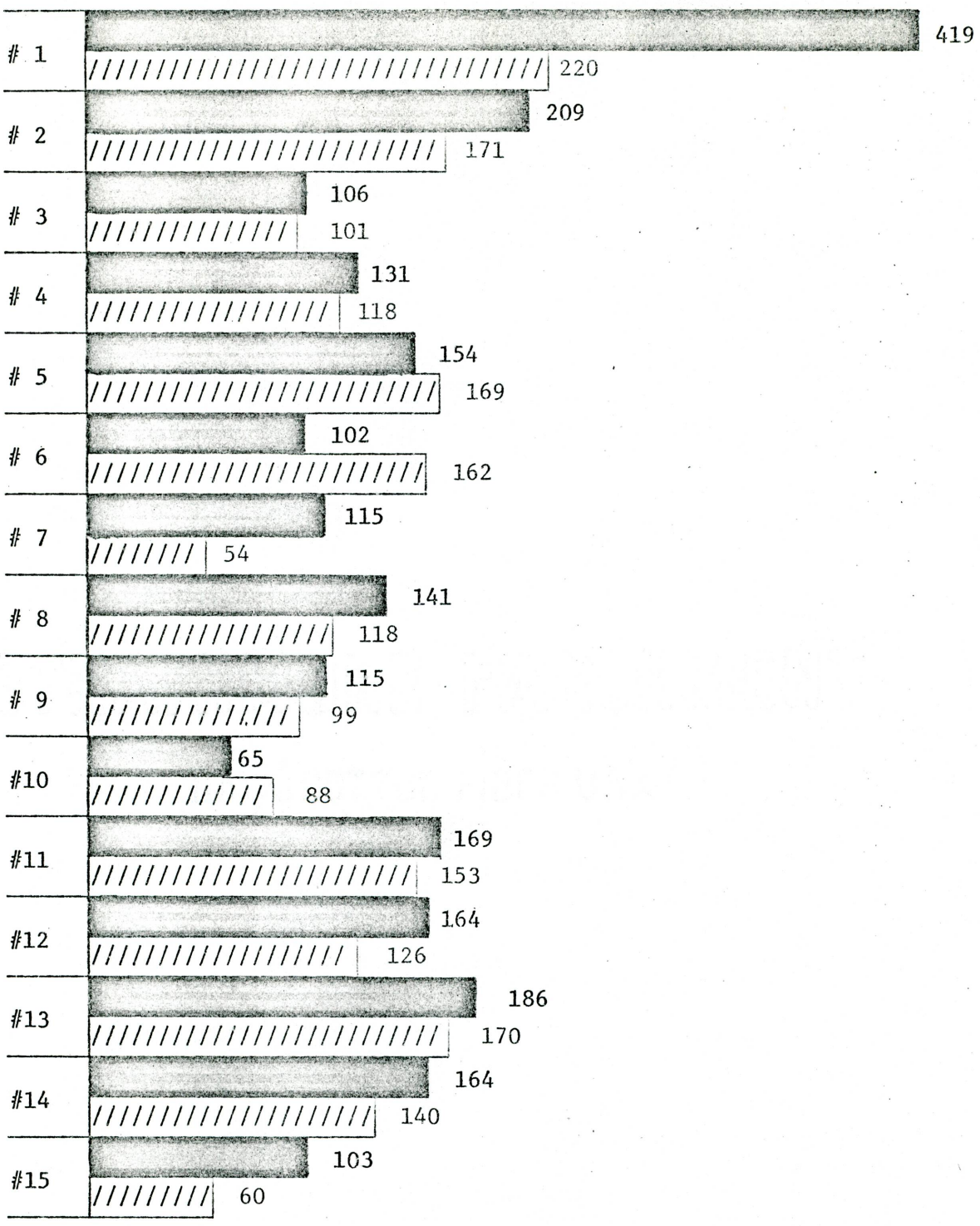

Figure 1. Serum triglyceride levels in mg.% before and after a high polyunsaturated fat, lacto-vegetarian diet.

Before diet ▭ After diet ////

*Serum triglycerides increased slightly.

During the four-week study there was a significant decrease in cholesterol levels ($p>.001$) (See Figure 2). The average decrease in cholesterol during this time was 78 mg.% with a mean drop of 29 per cent. Other studies show comparable, although not quite as great, results. A report conducted by McGandy et al. (1967) used a similar diet with fat providing 38 per cent of the calories and carbohydrate 45 per cent of the calories. At the end of four weeks, cholesterol had dropped an average of 10 mg.%. Another study by McGandy et al. (1967) reports a 12 per cent decrease in serum cholesterol with a diet restricted in saturated fats and high in polyunsaturated fats. Two more recent studies, using a cholesterol lowering diet with polyunsaturated fats, show comparable results. The Prudent American Heart Diet reduced serum cholesterol by 10 per cent and triglycerides by 12 per cent (Gotto et al., 1974), while the American Heart Association diet, given for six months, decreased serum cholesterol by 9.6 per cent and triglycerides by 11.7 per cent (Wilson et al., 1971).

A diet high in polyunsaturated fats tends to lower serum cholesterol because of its altered catabolic process. Polyunsaturated fatty acids undergo gamma oxidation due to the numerous double bonds. This results in the production of propionyl coenzyme A which is gluconeogenic and enters the Krebs Cycle at succinyl coenzyme A. In contrast, a saturated fat breaks down into two carbon fragments called acetyl groups which form cholesterol (Travis et al., 1972).

Although 12 of the 15 participants lowered serum triglycerides, the total effect of the high polyunsaturated fat diet was not statistically significant ($p<.05$) in lowering serum triglycerides. Of the 12

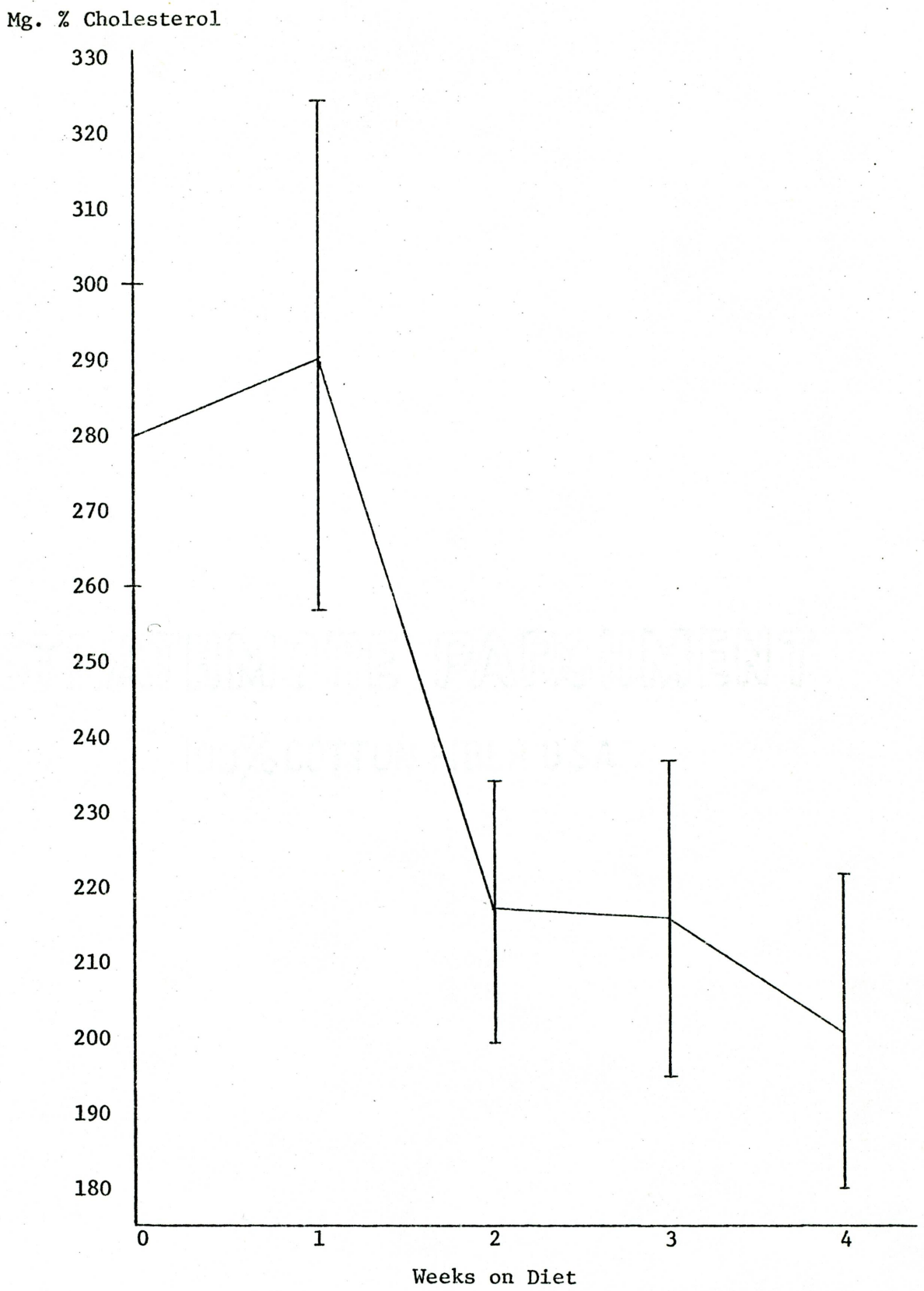

Figure 2. Mean serum cholesterol levels (with confidence intervals) taken at weekly intervals while subjects were on the cholesterol lowering diet.

men that experienced a reduction of serum triglycerides, the average decrease was 41 mg.%, the highest being 199 mg.% and the lowest 5 mg.% (See Figure 1). From results of similar studies, one notices some inconsistencies in the level of serum triglyceride drop. After one year on a cholesterol lowering diet, serum triglycerides fell by 17.3 per cent. Serum triglycerides fell significantly only in the men with hypertriglyceridemia (Hall et al., 1972). In a more recent study where high saturated fat and polyunsaturated fat diets were fed alternately, the men's serum triglyceride levels dropped by 35 per cent and serum cholesterol fell by 16 per cent (Chait et al., 1974).

Following the 28-day study, the subjects were allowed to follow any dietary pattern they wished. After six weeks time, a fasting blood sample was taken and serum cholesterol and serum triglyceride levels were determined. Nine of the 15 subjects had an increased serum cholesterol during the six weeks. The mean cholesterol for the whole group increased from 202 mg.% after four weeks on the diet, to 229 mg.%. During the six-week post-study period, 12 of the 15 subjects had an increase in their serum triglycerides. The mean triglyceride level for the entire group rose from 130 mg.% following the study to 171 mg.% six weeks after dietary treatment (See Table 4). While the triglyceride lowering effect of the diet was not statistically significant, it is of interest that the cessation of the diet caused an elevation of serum triglycerides.

A couple of the individual reactions to the diet are of interest. Subject #6, HGJ., is a perfect example of the effect of stress upon serum cholesterol levels. Following an illness which extended the length

Table 4

Serum Cholesterol and Serum Triglyceride Levels After the 4-Week Study and 6 Weeks on Their Own

	Triglycerides			Cholesterol		
	Initial	After 4 Week Study (mg.%)	6 Weeks After Study (mg.%)	Initial	After 4 Week Study (mg.%)	6 Weeks After Study (mg.%)
# 1	419	220	301	350	239	231
# 2	209	171	183	252	176	185
# 3	106	101	71	250	227	176
# 4	131	118	240	296	244	224
# 5	154	169	151	264	207	193
# 6	102	162	110	250	177	386
# 7	115	54	96	307	149	225
# 8	141	118	140	202	201	232
# 9	115	99	156	330	192	222
#10	65	88	110	307	204	237
#11	169	153	182	273	242	196
#12	164	126	132	270	251	224
#13	186	170	184	306	176	242
#14	164	140	368	257	176	249
#15	103	60	137	277	162	211
Averages	156	130	171	279	202	229

of the study, Mr. HGJ's wife died. Although he continued to decrease his intake of cholesterol and saturated fats, HGJ's serum cholesterol rose past the 300 mg.% mark and stabilized there (See Figure 4). Stress and anxiety seemed to play a major role in the elevation of this man's serum cholesterol.

Subject #8, B.L., received outstanding results from this study. As a result of elevated serum cholesterol, #8's personal physician placed him on four tablets of Atromid-100 approximately one month prior to the study. At that time, his serum cholesterol measured 304 mg.%. Although his values could not be used for statistical computation, a good contribution can be made with a comparison of Atromid-S's effect versus the high P:S ratio diet. Subject #8 gradually reduced his intake of medication so that by week 3 of the study, he was not taking any medication. B.L.'s cholesterol increased during the first two weeks, but as the diet became more of a routine, his cholesterol dropped. By the end of the study, #8 was able to maintain his cholesterol at normal levels (194 and 201 mg.%) without the aid of medication. During the six-week period at home, #8 followed a strict low cholesterol, low saturated fat, mainly vegetarian diet and was able to hold his serum cholesterol down to 232 mg.% without the use of Atromid-S (See Figure 1 and Table 4).

The high P:S ratio diet was expected to result in a lower serum cholesterol for all the participants. According to observations of Gotto (1973), with a 10 per cent drop in cholesterol, at least a 23 per cent decrease in coronary heart disease is expected. In this study, a 29 per cent decrease in the mean serum cholesterol should result in a greater than 23 per cent decrease in the risk of coronary heart disease for most of the men.

The 40 per cent decrease in serum triglycerides, although not significant, is quite meaningful. A study by Birchwood et al. (1970) shows that normal amounts of sucrose have no effect on serum triglycerides. Since sucrose was not limited in this study, it is safe to assume that any triglyceride lowering effect was due to the use of the highly polyunsaturated fat diet. Although the results were not statistically significant, they were important in that they further establish a relationship with high polyunsaturated fats leading to lower serum triglycerides. Much more study needs to be conducted, but evidence is mounting against using a strict low carbohydrate diet to combat elevated serum triglycerides.

SUMMARY

Fifteen male high risk coronary subjects were fed a lacto-vegetarian diet for 28 days. The diet results in a P:S ratio of 4.0 or greater and the subjects received at least 10 grams of fiber per day. At weekly intervals, the blood was drawn and serum cholesterol and triglyceride determinations were done. As a result of the diet, the serum cholesterol level decreased to a significant degree ($p>.001$) with a mean serum cholesterol drop of 29 per cent. The average decrease in cholesterol during the 28 days was 78 mg.%. Twelve of the 15 subjects experienced a reduction in serum triglycerides with a 40 per cent drop in mean serum triglycerides for the group, which was not statistically significant. Following the study, the men were left to a diet of their own choice for six weeks. During the six weeks, the mean serum cholesterol rose from 202 mg.% to 229 mg.% and the mean serum triglycerides rose from 130 mg.% to 171 mg.%.

Since carbohydrate intake was not restricted, the drop in triglycerides can be attributed to the lacto-vegetarian, high P:S ratio diet. This study supports the view that a low cholesterol, high P:S ratio diet is effective in reducing serum cholesterol and also strongly suggests that serum triglycerides may be reduced at the same time.

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APPENDIX

VERNIER RADCLIFFE MEMORIAL LIBRARY
LOMA LINDA UNIVERSITY
LOMA LINDA, CALIFORNIA

APPENDIX

INTERVIEW QUESTIONNAIRE

1. Name_____
2. Address_____
   _____
3. Phone_____
4. Age_____
5. Height_____ 6. Weight_____
7. Medications taken presently_____
   _____
8. Levels of (a) cholesterol_____
   (b) triglycerides_____
9. Blood pressure_____
10. Family medical history_____
    _____
    _____
11. Do you smoke? yes no ½ 1 2
12. Have you ever followed a special diet? yes no
    (a) restrictions_____
    _____
    (b) length of time followed_____
    _____
13. Is meat a regular item in your meal plan? yes no
14. Do you eat between meals? yes no
15. Frequency of between meal eating_____
    _____

In order to obtain scientifically valid data from this study, certain conditions are necessary:

1. Loma Linda vegetarian entree products will be served.
2. Three prepared meals will be served each day. Two in the dining area and one to carry out.
3. No meat is to be eaten.
4. Five blood samples will be taken during the 4-week test.

16. Would you be willing to follow this plan for 4 weeks? yes no
17. Do you have any food allergies?